

All stations measure wind speed, direction, and gust; barometric pressure; and air temperature. In addition, all buoy stations, and some C-MAN stations, measure sea surface temperature, salinity, wave heights and periods. See the website for more information.

Processing Steps:

1. Go to NDBC web site
2. Use buoy station map to identify name, location, and id of all buoys in northern California (north of Monterey Bay).
3. Input this info into a text file in the following format:

```
-----  
LATITUDE, LONGITUDE, ID, NAME, DESC  
41.85, -124.38, 46027, ST Georges, Northern CA NOAA National Data Buoy Station  
40.72, -124.52, 46022, Eel River, Northern CA NOAA National Data Buoy Station  
-----
```

4. Convert the list of stations to a shapefile in ArcView
5. Using the arcIMS hyperLink functionality (implemented in the arcIMSPARAM.js file) use the Station ID field to link station points in ArcIMS to data page on the CDEC website, like so:
hyperLinkLayers[2] = "NOAA NDBC Stations";
hyperLinkFields[2] = "ID";
hyperLinkPrefix[2] = "http://www.ndbc.noaa.gov/station_page.phtml?\$station=";
6. The hyperlink information is then used to launch related real-time data web pages on the NDBC website (e.g. [http://www.ndbc.noaa.gov/station_page.phtml?\\$station=46026](http://www.ndbc.noaa.gov/station_page.phtml?$station=46026))
Last updated 09/13/02

Example 2:

California DWR Data Exchange Center (CDEC) River Stage Data

URL is <http://cdec.water.ca.gov>

Attributes: stream flow, precipitation, humidity air temperature, river stage

Processing steps:

1. Go to CDEC Web site
2. Go to List of all Real-Time Reporting Stations, Sorted By Station Name (our list represents those stations last UPDATED: 05/03/2002).
<http://cdec.water.ca.gov/misc/realStations.html>
3. Save the list of stations as a textfile
4. Subselect only the stations in the following 4 counties:
Marin, Napa, Sonoma, Mendocino.
5. Reformat as textfile in MS Excel as comma delimited text file, with field names, like this:

```
-----  
Station ,ID ,Elev ,Latitude ,Longitude,County ,River Basin  
ARROYO CORTE MADERA MILL VALLEY,ACM,3,37.898,-122.535,MARIN,SF BAY  
-----
```

6. Convert the list of stations to a shapefile in ArcView
7. Using the arcIMS hyperLink functionality (implemented in the arcIMSPARAM.js file) use the Station ID field to link station points in ArcIMS to data page on the CDEC website, like so:
hyperLinkLayers[1] = "CDEC River Stage Data";
hyperLinkFields[1] = "ID";
hyperLinkPrefix[1] = "http://cdec.water.ca.gov/cgi-progs/plotReal?staid=";

(e.g. <http://cdec.water.ca.gov/cgi-progs/plotReal?staid=ACM>)

Last updated 09/13/02 by Patty Frontiera

Example 3:

USGS Stream Flow Data

URL <http://waterdata.usgs.gov/ca/nwis/>

Attributes: stream flow, water temperature, suspended sediments, river stage

The USGS NWIS Webdata website contains location and general information about ground water, surface water, and meteorological sites, including realtime data on current conditions transmitted from selected surface-water, ground-water, and water-quality sites.

Our shapefile of CA NWIS real-time sites on the BML-Salmon Site, represents those sites identified on the NWIS Webdata site on: 2002-07-24 14:05:51 EDT

Processing Steps:

1. Go to USGS NWIS webdata web site for California

<http://waterdata.usgs.gov/ca/nwis/>

2. Go to Real-time Data > Build table (<http://waterdata.usgs.gov/ca/nwis/current>)

3. Select "county" as the only site selection criteria and then click "submit"

(http://waterdata.usgs.gov/ca/nwis/current?search_criteria=county_cd&submitted_form=introduction)

4. On next form:

a) under the Select Sites section, select four counties (Marin, Mendocino, Napa, Sonoma)

b) Then, under "Choose Output Format" section, select "Site-description information displayed in Tab-separated format"

c) Under this, select the following fields: Agency, Site identification number, Site name, Decimal latitude, Decimal longitude, County code

5. Save the resultant web page as a textfile.

6. Reformat this textfile in Excel as comma delimited text file, for example:

SITENO,SITEID,STATION,LAT,LONG,CO_CODE

1,11460400,LAGUNITAS C A SP TAYLOR STATE PK CA,38.0269,-122.7353,41

2,11460600,LAGUNITAS C NR PT REYES STATION CA,38.0803,-122.7833,41

3,11460750,WALKER C NR MARSHALL CA,38.1758,-122.8172,41

4,11462500,RUSSIAN R NR HOPLAND CA,39.0267,-123.1294,45

7. Convert the list of stations to a shapefile in ArcView

8. Using the arcIMS hyperLink functionality (implemented in the arcIMSParam.js file) use the Station ID field to link station points in ArcIMS to data page on the CDEC website, like so:

hyperLinkLayers[0] = "USGS Streamflow Data";

hyperLinkFields[0] = "SITEID";

hyperLinkPrefix[0] = "http://waterdata.usgs.gov/ca/nwis/uv/?site_no=";

hyperLinkSuffix[0] = "&agency_cd=USGS";

9. The hyperlink information is then used to launch related real-time data web pages on the NDBC website, Example:

[http://www.ndbc.noaa.gov/station_page.phtml?\\$station=4602](http://www.ndbc.noaa.gov/station_page.phtml?$station=4602)

Projection Information

The shapefile created by the above process is in unprojected, geographic coordinates. However, the data served by ArcIMS for this project is in UTM Zone 10, NAD83. To resolve this, ArcIMS is used to reproject the shapefile on the file to UTM. Since it is such a small file, there is negligible performance issue.

Additional Information

See the file **codar_readme2.txt** on the CDrom for more details on the scripts used to process the codar data and installation notes. For information regarding the development of this site, contact Patty Frontiera at pattyf@regis.berkeley.edu or visit <http://www.regis.berkeley.edu>. For information on CODAR Ocean Sensors email support@codaros.com or visit <http://www.codaros.com>.

APPENDIX 3. RESPONSE TO REVIEWERS' COMMENTS

SCWA has taken the unusual step of soliciting three outside reviews of the final report for this contract, one from an academic researcher and two from private consultants. On the whole, these reviews laud the report as containing “very valuable research,” “impressive effort,” and “extremely useful information for resource managers.”

Our colleague, Dr. Bernie May, has provided the most useful technical comments and suggestions. We incorporate most of Dr. May’s specific suggestions (#s 4, 6-9, 11-12, 14-16, 20, 23) in this version of the final report. Other suggestions (3, 18, 19) will be addressed in the manuscript that will ultimately be submitted to a peer-reviewed journal. We did not incorporate the following specific suggestions for the reasons given:

1. The title of the contract used the word biodiversity, of which genetic diversity is an element.

2. We discussed several tasks, which did not yield results and were discontinued, in the 2001 annual report and in the main body of the final report. We chose to highlight the successful elements of the project in the Summary.

5. Objective 4 is simply re-stated from the original contract.

10. Moving Fig. 1 and Table 2 to the page following their first mention would make the Statistical methods section disjointed.

13. Bonferroni correction does not apply in this instance, since we are not performing multiple tests of the same underlying hypothesis across iterations.

17. The collection date of the smolts is the biological datum, related to different times of migration, as demonstrated by the genetic heterogeneity of the later outmigrants.

21. The Green Valley 1998 collections comprise a few large families ($n=25$, 15) and have relatively few unrelated individuals, so that the estimated N_b is less than N . In other juvenile samples, the smaller family sizes and relatively larger numbers of unrelated individuals, each one of which requires two parents, make $N_b > N$. In our judgment, based on experience with similar calculations for the Sacramento River winter Chinook salmon, the number and sizes of temporal samples would not support estimation of temporal variance with any precision.

22. We based our statement about the CCC and SSF ESUs on the significance of the nodes that unite all populations within these groups (nodes with bootstrap values of 618 and 914 in Fig. 8), which we interpret as support for the monophyly of the CCC and especially the SSF clades. The reviewer is questioning the significance of these groups based on the lack of support for the next deeper node in the tree. The ESU concept, however, is a statement about the similarity of populations within the unit, not a statement about the relative affinities of the ESUs to each other. Moreover, the lack of bootstrap support for the deeper node dividing CCC and SSF is caused, not by affinities of CCC and SSF populations but by spurious affinities of some CCC populations with some populations in the southern group of the SO/NC ESU.

The review from Chris Beasley et al., FishPro, deals mainly with methodological issues. The review often questions the biological relevance of the data and is especially concerned with sampling. We note that our study did concern threatened salmonid populations that are in low abundance. Still, by organizing and coordinating a large network of collectors from throughout the northern California region (this was accomplished primarily in the parent project to this contract), we were extraordinarily successful in sampling the small populations and ESUs of interest. We agree that sample size is an important consideration in a population genetic analysis, which is why the report devotes considerable attention to this issue. We give an especially detailed accounting of how coho salmon samples (listed in Table 2) are split, combined, or adjusted for kinship for further population genetic analyses. The objective of these procedures was to increase sample sizes for populations within and between drainages. That this was achieved is illustrated by comparing the distributions of sample sizes in the collections and the two subsequent data sets (Fig. A.3.1). A shift to the right in the modal sample size and a reduction in the number of samples having 10 or fewer fish are evident comparing the collections (blue bars) to the progressively adjusted data sets (yellow, then red bars).

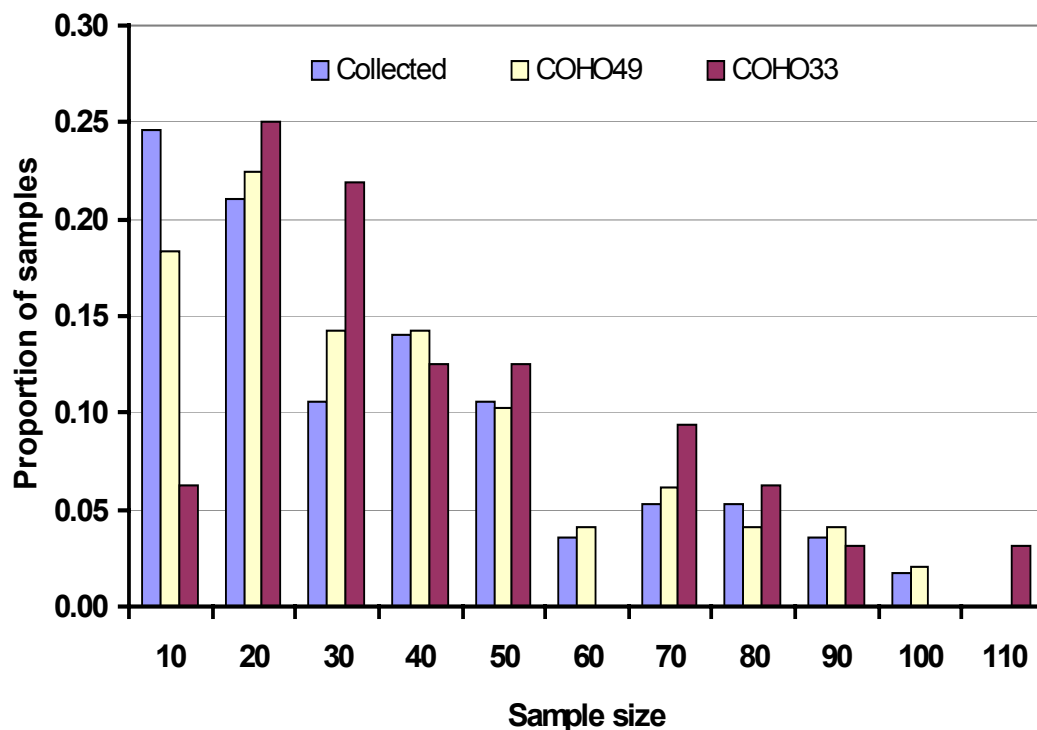


Fig. 1. Distribution of sample sizes in samples of coho salmon, as collected (57 collections, Table 2), after subdividing or dropping certain samples (COHO49, Table 3), and after further corrections for kinship and pooling of homogeneous samples within drainages (COHO33, Table 7; Lagunitas Creek [N=140] omitted).

We note that sample sizes of 20-50 individuals are typical for population genetic studies. As there are two alleles per individual, this number of individuals provides a sample of 40 to 100 alleles per sample, yielding sampling variances for an allele at a frequency of 0.2 between 0.004 and 0.0016, respectively. Finally, unbiased estimates of allelic frequencies can be obtained as long as sampling is done randomly with respect to age class and genotype. With the juvenile

samples, we show how to deal with non-random sampling of age-classes. Moreover, testing for departures from random mating expectations provides a compelling rationale for splitting or combining samples that is based on the extensive evidence that H-W equilibrium is observed in most natural populations of Pacific salmon.

In the statistical evaluation, the FishPro review seeks more rationale for the selection of markers, a discussion of the assumptions of SIBLINGS, and suggests the use of the program MIGRATE to determine effective population sizes and migration rates. As 57 of the 69 markers were eliminated either because they did not work or were not variable (Table 1), we were not compelled to give an elaborate rationale for rejecting those markers. We did select seven markers that seemed to show differences among California samples and rejected five that did not show promise for revealing population structure. The rationale and assumptions for the SIBLINGS program are elaborated in the Banks et al. 2000 reference cited. Estimation of migration rates and effective population sizes in natural populations is fraught with pitfalls. Assumptions underlying the classical method based on the relationship of $F_{ST} = 1/(4Nm + 1)$ are likely to be violated in the highly perturbed coho salmon populations of northern California (Whitlock and McCauley 1999). MIGRATE makes fewer assumptions but requires data for effective convergence of the likelihood simulation and cannot yet account for variation among subpopulations in effective size and growth rates.

FishPro's biological evaluation raises three issues, the effect of overlapping generations on temporal genetic variance, straying as a potential explanation of linkage disequilibrium in certain samples, and biological justification for binning population samples. Overlapping generations do confound the estimation of temporal genetic variance, though in a more complicated manner than the review suggests. Allele-frequency change between consecutive years overestimates mean temporal genetic variance in the population (Jorde and Ryman 1995), even when mean generational time is determined by a dominant year-class, as it is in coho salmon and the Sacramento River winter Chinook salmon (Churikov, Sabatino, Rashbrook, and Hedgecock, MS in preparation). Again, we did not attempt to estimate temporal genetic variance in any formal manner, because temporal sampling was not adequate. The comments about straying appear to confuse congruence between geographic and genetic distance with disequilibrium in juvenile samples, such those from Green Valley. Straying or admixture of adult stocks would not produce non-equilibrium proportions of genotypes in a cohort of progeny if mating among adults were at random. A Wahlund effect in a juvenile sample would only result from the admixture of progeny produced by different spawning populations, which seems unlikely in the case of the small Green Valley population but likely in other cases (*e.g.* WADY99, SCY99). Moreover, admixture would not produce significant kinship among pairs of individuals. To explain the congruence of geographic and genetic distance among samples, we hypothesize (not assume) that anthropogenic mixing has been ineffective and we propose an alternative explanation, as well. Finally, we partition samples when they show evidence of non-equilibrium genotypic proportions, when we have independent information, such as collection date or size, with which to partition them, and when the resulting sub-samples show equilibrium genotypic proportions. Likewise, we bin samples only when differences in allele frequencies among subpopulations are not significant and the resulting pooled sample conforms to random mating equilibrium proportions. With some samples (*e.g.* ESPRS99, MATS), we split and subsequently bin to differentiate the two causes of non-equilibrium. The justification for these manipulations is

genetic not ecological, but the results do suggest possible biological explanations (*i.e.* that fry emerging at different times or smolts emigrating from estuaries at different times come from genetically divergent populations of adults).

The FishPro review appears to agree with most of the interpretation of the coho results. We do think that Green Valley juveniles are a poor choice of brood stock for a hatchery restoration effort on the Russian River because they come from a very small number of adults and because Green Valley samples, even after adjustment for kinship, do not cluster with other CCC populations (Fig. 7). The congruence of geographic and genetic distance revealed by microsatellite DNA markers is not contradicted by the lack of single nucleotide polymorphisms in the coding genes examined by Kate Bucklin, since the mechanisms and rates of mutation in these two kinds of DNA are quite different.

FishPro raises similar concerns with respect to the Chinook salmon portion of the report. The Forsyth sample of eight adults was too small to stand on its own, so we pooled it with the nearest sample with which it was homogeneous, Mirabel 2000. Genetic distances among Russian River samples are less than 0.01, much smaller than the average distances of about 0.25 between samples from the Russian and Eel Rivers. The alternative pooling of Forsyth with the Mirabel 1999 sample would not alter this outcome. Likewise, temporal variation within the Eel River cannot explain the large divergence between Eel and Russian River samples. The 1998 and 1999 adults in the Eel River are homogeneous, even though temporal change between adjacent years should overestimate temporal variance in the population as a whole. We agree that temporal variation may be larger in the Russian River population than in the Eel River population, but the temporal variation that we have observed in the Russian River is much less than the spatial variation between drainages, a typical result in salmon population genetic studies. Contrary to the opinion expressed by this review, we feel that the samples of Chinook salmon analyzed in this study are sufficient to reach the conclusion that the Russian River stock has not been influenced by either Central Valley or Eel River stocks.

We are pleased that the third review by Ruth Sundermeyer, ENTRIX, Inc., finds that we have fulfilled our contractual obligations and contributed meaningful data. We respond briefly to some of her specific comments.

We do not agree that our summary statements or results “sum up to ‘We don’t know the effect of stock transfers.’” The congruence of geographical and genetic distance is difficult to explain in the face of concerted efforts to transfer stocks among drainages, basins, and states. Generally, concordance of geography and genetics is achieved over evolutionary time scales. Whatever stock transfers have occurred (and we agree that a detailed account of these would be helpful were it available), they have not erased the phylogeographic pattern. We do entertain the not mutually exclusive hypothesis that drift in small populations may have kept pace with anthropogenic homogenization, but we believe that this explanation is not as likely as the first.

Whether divergence among populations at microsatellite DNA markers reflects the forces of natural selection or random genetic drift in a network of incompletely isolated populations cannot be determined in most population genetic studies and may not be necessary to resolving population units for conservation. We believe that our results support the ESUs identified by the

California ESA, which recognizes a subdivision between the CCC and SSF units. A deviation from H-W that disappears upon partitioning of a sample according to independent information is a clear reflection of population subdivision whether this is caused by adaptive divergence or drift.

We welcome the information regarding the history of the Waddell Creek and Scott Creek coho salmon populations, which may provide insight into their genetic affinities. It is unfortunate that this information has not been published in peer-reviewed journals.

Reference

Jorde, P. E., and N. Ryman. 1995. Temporal allele frequency change and estimation of effective size in populations with overlapping generations. *Genetics* 139:1077-1090.

APPENDIX 4. REVIEWS

Reviews of the penultimate draft of the final report that were solicited by SCWA are attached. The three reviews are from:

Dr. Bernie May, UC Davis
Chris Beasley, FishPRO,
Ruth Sundermeyer, Entrix

**Review of Final Report “Documenting Biodiversity of Coastal Salmon
(*Oncorhynchus* spp.) in Northern California by Hedgecock et al. Dec. 2002**

by Bernie May (Jan. 10, 2003)

UCDavis

General Comments

1. Above all this report describes very valuable research in salmonid genetics, with special relevance to those of Northern California. SCWA clearly received their money's worth over the past five years from the efforts of the Hedgecock laboratory. The comments detailed below are queries and suggestions for the overall improvement of this final report.
2. In general the report is very clear and well organized, although the text features (capital letters, boldface, underlining, italics, etc.) used to indicate hierarchy of headings and subheadings can be confusing. Hierarchical numbering would improve the readability of the text and use of the TOC.
3. Good explanations of basic genetic principles like H-W equilibrium and F_{ST} are given and used effectively throughout the report. If any of this material is still unclear to SCWA, I encourage them to ask for further clarification. This is a great opportunity to get genetic concepts explained.
4. The use of their computer program Kinship to remove the bias of related individuals from juvenile samples is well explained. Removing this bias is a significant contribution by this group of investigators to population genetic studies of any organisms.
5. The report does an excellent job of addressing the objectives outlined in the SOW with a few exceptions. The section on “Alternative male-types” was underdeveloped. The attachment of three vague abstracts provides limited useful descriptions of the experiments and results. In the objectives for the coho study it is stated that protein-encoding gene and mtDNA markers will be developed as well as microsatellite loci; why this was not done should be mentioned more extensively. Also in the coho objectives, it was stated that estimates of migration rates among and effective population sizes of spawning runs would be given, although only the effective population size of the green

Valley Creek samples are presented. Some of these issues were resolved in the 2001 report, but probably should be repeated in this Final report since they are pertinent to the SOW.

6. A final overall section on “Future Needs” or “Recommendations” should probably be added that are directed toward SCWA and not simply scientific interest.

Specific Comments

TITLE:

1. While “biodiversity” was used in the SOW, the results described are primarily about salmonid “genetic diversity”.

SUMMARY:

2. Steelhead work is mentioned in the introductory sentence but not discussed further in the summary. The discontinuance of the several portions of the SOW (e.g., steelhead work, historical samples, mtDNA, phylogeny) should be mentioned in the summary.
3. Possible admixtures are offered as an explanation for the deviations from random mating equilibrium. How do you distinguish the effects of admixtures from the effects of stock transfers?
4. “Diversifying effects of genetic drift” (bottom of 1st full paragraph on page 3) – awkward wording since you associate genetic drift with a loss of heterozygosity. Perhaps 3 and 4 could be addressed in the discussion of genetic principles.

POPULATION GENETICS OF COASTAL CALIFORNIA COHO SALMON

POPULATIONS:

Introduction

5. In the list of objectives, number 4 would be better split into two objectives.
6. One of the conclusions of the report is that the genetic data corroborates the previously established ESUs for Coho the U.S. Pacific coast. While it is certainly beyond the scope of the report to describe how the ESUs were established in the first place, it would be

nice to see a reference to the document (possibly a Federal Register) that describes the ESUs for Coho. Additionally a map showing the geographical distribution of the ESUs would be helpful.

Materials and methods

7. Page 5 fix wording so as not to use " $(p+q)^2=Np^2+2Npq+Nq^2$ " which is not mathematically correct.
8. Pg. 6- last paragraph; the following sentence describing F_{ST} could be more clear. "The genetic correlation between gametes drawn from different demes or subpopulations, with respect to the allelic frequencies in the total population, is given by F_{ST} , the ratio of the variance of allelic frequencies among subpopulations to the maximum." Perhaps the above sentence would be clearer if the term "maximum" were replaced w/ the phrase "variance in allelic frequencies among all subpoulations".
9. Fig. 1 legend should indicate that sample sizes are found in Table 2.
10. Fig. 1 and Table 2 should be included in the text after page 12 (the page where they are first mentioned).
11. Page 8 under "Microsatellite DNA markers": The text states that there were 67 microsatellites for testing, while the table refers to 69.
12. Top of page 13: remove the parentheses around the decimal values... otherwise, it appears that you're multiplying. Try "1/10 or 0.1"
13. In determining significance of F_{ST} values multiple tests were done iteratively by testing a group of populations, removing the most divergent one, and then retesting the group until none of the remaining populations were significantly different. Some discussion of adjusting P values (i.e. Bonferroni corrections) for this kind of testing should be included, since adjustments would greatly effect which populations are excluded and included from the final set.
14. Define LOD abbreviation on page 14.
15. Page 15, KIGHA description: N=15, not 13.
16. Page 16, ESPRS99: Should the populations be 68-92 and 96-110?

17. Page 17, MATS: What biological data supports the initial division of the samples collected 5/7-5/11 and the samples collected 5/12-5/16?
18. Page 18, RRGV98b: It might be of concern that some of the same individuals from 98a may be represented in the 98b population. Was a test for homogeneity of these two populations performed? It's interesting that both 98a and 98b have the same proportion of loci pairs showing significant associations.

Results

19. Color coding (or alternate acronyms) of populations by ESU/river in Figs. 6-8 would help the reader see the geographic relationships presented.
20. Table 3: Does NA signify no amplification?
21. An estimate of the number of breeders was done for the Green Valley samples. Why wasn't this done for any other populations to see if genetic drift can explain the random mating deviations? Alternatively, why were the temporal variation data not used to estimate N_e ?
22. Page 30, last paragraph: It is stated that the phylogenetic trees correspond to the designated ESUs. However, the CC and SSF clusters is not supported by the bootstrap analysis. Therefore, I am unclear how the distinction between those 2 ESUs can be confirmed.
23. Pg. 30- I believe they mean Fig. 8 and not Fig. 9 in the last paragraph on this page.

Discussion

24. Under Departures from random mating equilibrium in CA coho salmon populations, third paragraph, it is suggested that the Wahlund effect probably doesn't explain the observed deviations from random mating equilibria due to small spatial and temporal scales involved. Give examples and references that show temporal and spatial homogeneity in other salmonids populations (some researchers have shown significant heterogeneity on small spatial scales (i.e. within tributaries) for east coast U.S. Atlantic salmon).

25. Under Departures from random mating equilibrium in CA coho salmon populations, fifth paragraph, some examples with references regarding temporal genetic heterogeneity would be helpful.

STOCK ORIGIN ESTIMATES FOR CHINOOK JUVENILES CAPTURED IN THE RUSSIAN RIVER

Results

26. Results are written in the present tense, while results of the coho work were presented in past tense.
27. Page 40, Results: Table 10 should be referenced, not Table 2.
28. Since there are no historical samples of Russian River fish, derivation of the current Russian river fish seems unknown. The possibility for inclusion of some hatchery component would still seem feasible. It would seem that more coastal chinook populations should be included in an analysis before any conclusions are drawn.

DEVELOPMENT OF GEOGRAPHIC INFORMATION SYSTEMS

29. Testing a GIS mapserver was interesting. However, I was unable to use the web site at all. Even trying to retrieve a document gave the response “The document that you have requested is currently being processed or updated. Please check back later.” Use of these technologies to map genetic data with biogeography will certainly extend our understanding of the effects of environmental variation on the numbers of salmonids and consequently genetic variation. The discussion is clear and extensive about the value of these tools and ways they can be developed and extended. However, this is a general scientific discussion. Specifically, what should SCWA do now? Should they be involved? How should they be involved? Who will maintain the existing mapserver?

Final Note: This is an excellent example of a report from a university investigator to a funding agency. It is unusual to have these reports reviewed. That being said, such reviews should be common occurrences.

Review of Hedgecock et al. 2002:

**Documenting Biodiversity of Coastal Salmon (*Oncorhynchus* spp.) in Northern California,
Final Report, December 2002**

Prepared by:
FishPro, a division of HDR
Chris Beasley, principal author

PART I: COHO SALMON ANALYSES

Introduction

In general the researchers have made an impressive effort to elucidate the relationship among coho salmon spawning aggregates in California. That being said, a more detailed account of sampling effort is desperately needed. Throughout the report, the authors note that only juvenile samples were available from some spawning aggregates, and in many cases, the total sample size of those juvenile groups was small. In order to determine whether the results of the analyses are biologically meaningful, the reader must know if sample sizes and sampled life history stages were limited by sampling effort or actual abundance. For example, were temporal replicates from spawning aggregates not available because no one attempted to collect them, or because fish were not encountered after exhaustive sample efforts? Given this limitation, the only meaningful comments that I can make regarding this research are methodology based.

I have grouped comments into three categories: statistical, biological, and interpretation of results.

Statistical Evaluation

The researchers have employed several methods to statistically compensate for small sample sizes and less than ideal distributions of sampled life history stages. However, there are some details that could be profitably expanded upon. For example, a general discussion of marker selection, the assumptions of some of the methods, as well as the potential for utilizing alternative analyses should be included.

The researchers screened some 67 microsatellites from which they selected seven for use in their study of coho salmon. This represents a substantial effort, however it is not entirely clear why some loci were excluded, or why only seven loci were selected. I am not suggesting that the selected loci were insufficient, only that some discussion of the statistical value of the selected loci as well as the tradeoff between using highly polymorphic markers versus markers with less polymorphism is warranted. Was the selection of markers tailored to the methods that the authors intended to use, or were analysis methods selected *post hoc*? In general however, I agree that the selected markers exhibit a degree of polymorphism well matched to the sample sizes that were available, with the possible exception of *Ots-103*, for which the number of alleles is larger than the sample size for some groups.

The use of the programs KINSHIP and SIBLINGS allowed for a much more statistically rigorous examination of the genetic relationships of individuals within subgroups, and hence

allowed for potentially more robust tests of differentiation/relatedness of subgroups and spawning aggregates. However, a discussion of the assumptions required by SIBLINGS, as well as the potential biases introduced by the use of this program is lacking.

Beyond the use of the program SIBLINGS, the analysis of the resulting hypothetical parental and unrelated samples is straightforward and sufficient for the purpose of the report. However, there are some additional analytical tools that might aid in interpretation of the results. For example, the program MIGRATE (Beerli and Felsenstein 2001) could be used as an alternative method for computation of effective population sizes, and additionally could be used to estimate rates of migration between spawning aggregates. For many of the key questions it might be useful to use a number of methods, each with different assumptions, to calculate the quantity of interest. Doing so would erase any doubt that a given result is method dependent and indicate whether results are robust regardless of the methodology used and the assumptions required (and potentially violated).

Biological Evaluation

Some basic life-history characteristics of coho could be discussed in more detail. For example, throughout their range, coho exhibit a relatively fixed three-year life cycle. So, one might expect greater temporal variation between repeated samples from a coho spawning aggregate than for other Pacific salmonids (e.g., chinook salmon) for which overlapping generations might have the effect of decreasing temporal variation within a spawning aggregate. While this feature of coho salmon life history is probably not an adequate explanation for the F_{ST} values observed between temporal samples from some of the spawning aggregates, it could explain at least a portion of that variation. This is particularly true for the “jack” versus adult samples analyzed from the Trinity River Hatchery.

Regarding the congruence of geography and genetics, the authors should consider a discussion of documented straying among coho spawning aggregates from the Pacific Northwest (rather than dismissing the potential contribution of strays to a Wahlund effect – the stated alternative to inbreeding as an explanation for high rates of observed linkage disequilibrium). This is a particularly important point for Green Valley samples, given the history of different broodstock sources potentially introduced to this area from hatchery programs. Additional ancillary data suggest that the assumption that decreased fitness of hatchery stocks may have precluded their contribution to natural production may be faulty. For example, in the Columbia River Basin, reintroductions of coho salmon in the upper Columbia and Snake Rivers, using downriver hatchery stocks, has been enormously successful, suggesting one or more of several alternatives; that coho exhibit a remarkable degree of plasticity, are not as prone to large fitness differentials resulting from local adaptation, and/or are not as greatly effected by hatchery rearing as other species of Pacific salmon. However, these data are available only in the form of “white” papers, and hence are not part of the accessible pool of peer-reviewed literature.

Finally, in an attempt to minimize linkage disequilibrium, the researchers decomposed sample groups into a number of subgroups, some of which were clearly justifiable, but others that are biologically questionable. For example, separating “jack” coho from other adult coho at the Trinity River Hatchery has a biological basis, given that they are of different cohorts, and hence

arise from temporally isolated parental populations. Alternatively, it is not at all clear that binning samples based on capture date from the lower South Fork trap on the Little River is biologically justifiable (it may be, but some justification is required). The same is true for the ESPRS99 sample group (which was subdivided based on a size gap from 92 to 96 mm, which could potentially be explained by a few days difference in emergence and growth) and the MATS sample group (subdivided based on apparently arbitrary binning of capture dates). Again, there could be credible biological justification for these subdivisions, but it is not provided.

Interpretation of the Results

Overall, it is my opinion that the researchers present an unbiased interpretation of the results, although the management ramifications of their interpretations could be discussed in greater detail. I agree that the data indicate that the Eel, Russian (Green Valley), and Noyo River samples all exhibit high within group relatedness (e.g., high probability of inbreeding), and that this interpretation is a more plausible, and better supported, explanation for observed linkage disequilibrium than the Wahlund effect. In general, sample groups exhibited high levels of relatedness, however the authors' assertion that inbreeding depression may be contributing to the decline of the species cannot be directly addressed with these data without ancillary information.

I agree with the authors that the Green Valley coho spawning aggregate exhibits a high degree of relatedness, thus the authors rightfully express concern regarding a program that derives broodstock solely from a (apparently) highly inbred population. However, I am confused by the parting statement that "this small population appears to be anomalous and unrepresentative of the Central California ESU." What is intended by this comment? Does its distinctiveness make this spawning aggregate critical for conservation, or are the authors suggesting that recovery efforts for this "anomalous" population should be abandoned?

Conclusions

The stated objectives for this research included: 1) to determine relatedness in samples comprised of juveniles; 2) to determine temporal genetic variation within year classes; 3) to estimate genetic divergence among and effective population sizes of spawning runs; 4) to determine genetic change between historical and extant coho populations; and 5) to relate the genetic diversity of California coho populations to environmental and biological factors being measured in the sampling process. In general, objectives one, two, and three were satisfied, with the exception that available samples limits my faith in the interpretation of results, and with the exception that effective population size was dealt with explicitly only for the Green Valley sample group. Objective four was not addressed, and could not be addressed with the samples that were analyzed. Objective five also was not addressed, but should be given that the interpretation of results relies on the ability of the samples to represent biological reality.

Some puzzling statements were included in the report. For example, the authors suggest that the results support existing ESU designations, but this assertion appears inconsistent with the research completed by Kate Bucklin that found "very little" variation at the nucleotide level. Perhaps more detail could explain the potential discrepancy.

Finally, while it is arguably outside the scope of the contracted work, the authors undoubtedly are in the best position to suggest management alternatives from a genetic standpoint. While management decisions for California coho cannot, and should not, be based solely on genetic considerations, the researchers could provide more directed management guidance. A statement relaying the faith that the authors place in the results would be helpful.

PART II: STEELHEAD ANALYSES

Apparently none of the objectives for steelhead were completed. In addition, the proposed research into candidate genes controlling run timing, while interesting, would have been unlikely to address all listed study objectives.

PART III: CHINOOK SALMON

Introduction

Similar to the review of coho analyses, I have structured comments regarding the analyses pertaining to chinook salmon as statistical, biological, and interpretation. Also, as with the coho analyses, it is unclear why sample sizes and replication of sample groups is less than desirable. Two of the largest sample groups (Warm Springs Hatchery adults samples from 1997) were excluded from analyses based on high rates of observed linkage disequilibria. Are the authors comfortable that the samples made available for analysis adequately represent genetic variation, and hence can be used as a basis for biologically meaningful management decisions?

Statistical

The analyses employed by the researchers to address genetic variation and differentiation among the sampled groups of chinook salmon would be acceptable if sample sizes and temporal replicates were available. However the sample sizes available, and the temporal distribution of those samples decreases my faith in the resulting analyses (see “biological” comments). The researchers emphasize that the resolved Russian River samples cluster together (bootstrap value of 848/1000), but are “distinct” from Eel River samples (bootstrap value of 919/1000), this is a problematic statement for two reasons.

First, given that the Forsyth 1999 sample group was not significantly distinguishable from either Russian River sample group it is not clear why the Forsyth samples were grouped with the Mirabel 2000 samples (why not group them with the Mirabel 1999 samples, or better yet treat them independently?). In this case it might be useful to review the sources of genetic sampling error that might obscure comparisons. Within the Russian River samples for example, eight adult samples from 1999 were grouped with 82 juvenile samples from 2000. We might expect that such a grouping would potentially increase genetic distance between the 1999 Mirabel juvenile sample and the combined 1998 Forsyth adult/2000 Mirabel juvenile sample. In essence, temporal variation might be introduced by the grouping of juvenile and adult samples, as well as grouping samples from different geographic locations (Mirabel versus Forsyth). Although both sources of variation were shown to be insignificant when treated independently, it is possible

that the respective errors, when treated concurrently by grouping samples, could contribute to the perceived distinctiveness in further tests (e.g., Mirabel 2000/Forsyth 1999 versus Mirabel 1999). Unless there is a good reason to group the Mirabel 2000 and Forsyth 1999 sample, I would recommend treating them separately.

Second, adults from the Eel River are being compared to juveniles (and a few adults) from the Russian River. Aside from the fact that the authors suggest this is less than ideal situation; at least in regards to the coho analyses, there are reasons to believe such a comparison for these samples is problematic. Foremost, there is potential for temporal variation to obscure the relationship between Eel River adults and Russian River juveniles. Eel River adults likely arose from spawning in 1993 through 1996, while Russian River juveniles likely arose from spawning in 1998 and 1999. Up to six years of genetic drift (likely exacerbated by small and declining population size) separates these sample groups. While the Eel River spawning aggregates appear to be temporally stable, as evidenced by non-significant differentiation between adults sampled in 1998 and 1999, such stability is apparently not exhibited by Russian River spawning aggregates, as evidenced by significant temporal variation between smolts sampled from Mirabel in 1999 and 2000. How much of the perceived differentiation between these groups could be assigned to temporal variation?

In short, the researchers likely embarked on a series of exploratory analyses that led them to group samples for the final analyses presented in this report. For the reader, it might be useful to show the results of these analyses, or to describe them in more detail. Finally, I would recommend that the authors present a Cavalli-Sforza and Edwards UPGMA dendrogram that shows the genetic relationship of ungrouped samples. If the overall result (differentiation between Eel and Russian River samples) is supported by analysis of ungrouped samples, interpretation would be more straightforward.

Biological

Regardless of statistical methods employed, the utility of genetic analyses relies on how well samples represent a population of interest. It is not at all clear that the chinook salmon samples used in these analyses are capable of expressing the range of temporal and geographic variation that is exhibited within and among the sampled spawning aggregates. As mentioned above, the most biologically troubling aspect of this analysis is the comparison of temporally distant samples from the Eel and Russian Rivers.

Interpretation

The major finding of this research is that chinook salmon collected in the Eel River appear to be distinct from chinook salmon collected in the Russian River. Whether this result is biologically meaningful remains questionable.

Conclusion

This report was intended to address two tasks: 1) establishment of a genetic baseline for chinook salmon populations from Mendocino and Sonoma counties, and comparison of those spawning

aggregates to known spawning aggregates and 2) to determine the relationship between Russian River and other coastal chinook spawning aggregates including both extant and historical population samples from drainages such as the Eel River. The second task was apparently not addressed by this research, and the first task, while underway, in my opinion is incomplete. It would be helpful if the authors provided a definition for what constitutes a “genetic baseline.” My definition, which is only one interpretation, would be a series of samples which when analyzed would yield an estimate of the temporal and geographic variation exhibited by chinook spawning aggregates within a watershed of interest. To construct such a baseline would require temporally repeated samples (ideally taken annually for an entire generation) from geographic locations within a watershed known to support spawning. Comparing such baselines from a number of watersheds would allow a robust construction of the relationships between stocks on a larger geographic scale. Data presented in this report are therefore a good start to such a baseline, but fall short of my definition. As such, the conclusions reported by the researchers are potentially effected by some unknown degree by temporal and/or geographic variation that may not have been measured.

OVERALL CONCLUSIONS

The objective of this review was to determine whether contractual obligations were satisfied by this research. With regard to coho salmon, I would say that the researchers have satisfied their obligation, with the caveats mentioned below. Steelhead objectives have not been achieved, at least as presented here, nor have the objectives for chinook salmon been achieved. Each of the previous statements, however must be bounded by the caveat that a laboratory can only analyze the samples that are received. In general, this research, and the conclusions that can be drawn from the analyses, is severely limited by sample availability.

Aside from sampling considerations, this report would greatly benefit from increased detail regarding the researchers faith in the results. Are the authors convinced that the results of their analyses are biologically meaningful, or do they feel that the results are constrained by sample availability to a degree that limits their utility for management? Given that the researchers are in arguably the best position to provide management guidance from a genetic perspective, it would be useful (and a departure from the ordinary) for them to include a section detailing their respective opinions regarding management.

Finally, the GIS applications as well as the research into alternative male coho phenotypes was not linked to specific contractual obligations – despite the fact that both of these data types could be potentially provide guidance to data analysis.

Literature Cited

Beerli, P. and J. Felsenstein (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. PNAS 98(8): 4563-4568.

Review of Hedgecock *et al.* 2002 Documenting Biodiversity of Coastal Salmon (*Oncorhynchus* spp.) in Northern California

By Ruth Sundermeyer, ENTRIX, Inc., January 2003

Summary

This review of Hedgecock *et al.* 2002 includes comments on the report's scientific merit, methodology, fulfillment of contract terms and conditions, and literature review.

Hedgecock *et al.* 2002 provides extremely useful information for resource managers as they make decisions in resource and recovery planning. Furthermore, the study is written in such a way that should make it easier for managers and biologists who are not geneticists to not only understand the results of the study, but to understand how this information can and can not be used. It will be important for people with local knowledge within their watershed to help interpret the genetic information presented so that informed management decisions can be made. One of the strongest features of the study is the statistical tools developed to address factors that are important to consider when interpreting genetic results, in particular, correction of juvenile samples for sibling relationships. When working with threatened or endangered species, assumptions of hypothetical models or statistical analysis packages can be difficult to meet, and the researchers on this study have made important contributions to the resolution of some of these issues.

Fulfillment of Tasks in the Contract

The major objective of the contract, to describe the genetic diversity of the coho salmon populations along the central and northern coast of California, was fulfilled. Specific tasks that were fulfilled included 1) to determine relatedness in samples comprised of juveniles, 2) to determine temporal genetic variation among year classes and 5) to relate the genetic diversity of California coho populations to environmental and biological factors being measured in the sampling process. Task 3) to estimate genetic divergence among and effective population sizes of spawning runs, was mostly completed, but effective population sizes of spawning runs were not determined for all populations for which this information might be useful. Task 4) to determine genetic change between historical and extant coho populations to assess influence of hatchery plantings and reductions in abundance, is a difficult task that may never be completely resolved. Furthermore, an historical collection free of hatchery influence would be difficult to compile, given the extensive and incompletely documented stocking history.

Tasks in the Chinook portion of the contract were fulfilled.

Specific Comments to Text

Page 3, Summary, end of 2nd paragraph.

"The congruence of genetic and geographic distance is surprising in light of the history of coho stock transfers within California and between California and other Pacific Coast states."

However,

"Stock transfers appear to have left no genetic mark on extant populations. Alternatively, or in addition to stock transfers, the diversifying effect of genetic drift within the relict coho populations of California may be keeping pace with whatever homogenization has been or is being affected by hatchery practices."

These two sentences sum up to 'We don't know the effect of stock transfers.' The analysis presented in this report does an excellent job of outlining existing population structure, but without an accurate, historical baseline and a more through review of past hatchery practices, analysis of effects of stock transfer remains speculative. It is hoped that resource managers

familiar with local stocking histories and local ecological factors will be able to apply their knowledge to help refine the interpretation of the findings presented in this study.

Page 4 Last paragraph.

This is a good summary to help show how F_{ST} can be interpreted. It might be helpful to add a point about local adaptation as a potential factor for diversity between populations. An artificially induced deficiency of heterozygotes is a different kind of management problem than genetic structure and amount of genetic diversity influenced by natural selection, which may have a direct effect on fitness of the population. Furthermore, population structure that is based on natural selection can change if the adaptive landscape changes, such as large changes in weather patterns associated with extremely wet years and El Nino events, and may also cause changes in H-W and F_{ST} . Although this is a difficult question to answer, conceptually it may be important to when trying to make management decisions based on genetic analysis. A thorough understanding of historical hatchery practices and local ecological data are essential, especially when one is trying to identify remnant “natural” populations for protection.

It can be difficult to know at what scale management of differentiated population units should occur – one can micromanage populations on too fine a scale within a watershed, or manage on a scale that does not protect local adaptations.

Page 14.

“Wahlund effect in the original sample would be evidenced by non-significant departures from H-W within subsamples, but significant F_{ST} among subsamples.”

Are there no other possibilities besides an artificially induced Wahlund effect? Subsamples collected from different sites within a watershed – couldn’t they be in H-W equilibrium and have significant F_{ST} if admixture occurred with a change in the adaptive landscape other than artificial stocking?

It should be noted that the analysis on subgroups within samples based on information such as year class, size, and geographic information is information that is not routinely presented in studies of this kind, but can be very useful to interpret the population structures observed. It may also help identify admixture due to hatchery influences.

Page 19 Waddell and Scott Creeks

Waddell and Scott creeks provide an interesting case study because there is a long-term juvenile abundance data set for Scott and Waddell creeks spanning almost a decade, as well as relevant ecological information and stocking history that help explain population trends (Smith 2002).

WADY99: *“Samples originating from RM 4.7 were heterogeneous to both RM 3.1 and 3.9 and were removed (WADY99up).”*

SCY99: *Removal of Upper Fork and RM 4.9 samples (SCY99up) yields a homogenous population (SCY99low) with a substantial number of siblings.*

Big Creek hatchery (from the Scott Creek watershed) coho fry were planted in lower Waddell in 1996, (perhaps progeny of SCA95), but not in upper Waddell. Juvenile fish from the 1996 year class in Waddell would have spawned the 1999 year class, including WADY99up and WADY99low, while juvenile fish from the strong Scott Creek 1996 year class would have contributed to the SCA98 and SCY99 samples. This hatchery stocking might help explain why WADY99low is more closely related to SCA9798 (F_{ST} 0.019), SCA95 (F_{ST} 0.014) and SCY99low (F_{ST} 0.017) but WADY99up is more distant to these same populations (F_{ST} 0.076, 0.074, and 0.041 respectively). It also appears that SCY99up is more closely related to SCY99low (F_{ST} 0.024) than to WADY99up (F_{ST} 0.120).

The survival of naturally spawned juvenile fish is more certain in some sections of Waddell and Scott creeks than in others depending on winter storms and summer flow (Smith 2002) and it would be interesting to see if the population structure documented in this study persists. The 1993, 1996, 1999 and 2002 year class set (a set that spans the three year life history of coho) is currently the only viable set in Waddell (Smith 1999, 2002), which makes the WADY99 populations (and 2002 juveniles) important. Smith 2002 describes the status of coho in several streams in the south of San Francisco group in detail.

Page 36 Temporal Variation

Although temporal samples are available for seven sites, additional information is needed to interpret some of these data.

In Scott Creek, presumably the SCA95 population contributed to the SCA98 population. In 1993, a strong juvenile year class was documented (Smith 2002) and precocial females from that year were raised in the hatchery to supplement weak year classes. This suggests that the Scott Creek populations examined in this study may be a better case study for artificially induced year-to-year variation than for natural temporal variation. Furthermore, the persistence of weak year classes could contribute to the genetic variability between year classes, and tests for inbreeding would be helpful.

It would be helpful to examine hatchery stocking and natural factors affecting population trends in other watersheds as well. For example, hatchery planting may contribute to homogeneity of samples between locations and years, such as is found in Lagunitas. The surprising heterogeneity between the Redwood Creek samples (RWMA97 and offspring RWMY98), as well as the fact that they are outliers is a mystery that draws curiosity.

Page 42 Discussion on Chinook results

“Chinook in the Russian River do appear to belong to a diverse set of coastal chinook populations.”

Although it is useful to know that Russian River chinook are not closely related to Central Valley or the Eel River chinook, the relationship to other populations in this ESU can not be determined without comparison to additional data, such as hatchery populations from which extensive stocking in the Russian River has occurred. In any case, this may be a difficult question to answer, given the long history of stock transfers in this basin.

References

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